PSEUDOMYCIN PRODRUGS

FIELD OF THE INVENTION

The present invention relates to pseudomycin compounds, in particular, prodrugs of pseudomycin compounds.

BACKGROUND OF THE INVENTION

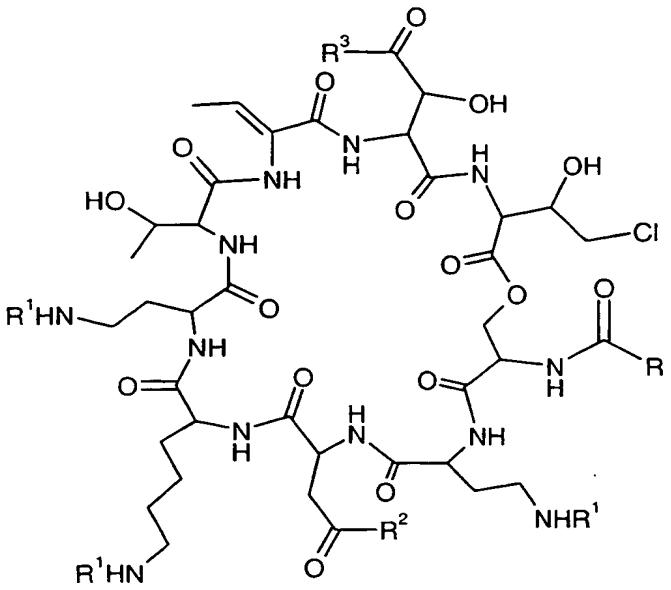
Pseudomycins are natural products isolated from liquid cultures of *Pseudomonas syringae* (plant-associated bacterium) and have been shown to have antifungal activities. (see i.e., Harrison, L., et al., "Pseudomycins, a family of novel peptides from *Pseudomonas syringae* possessing broad-spectrum antifungal activity," J. Gen. Microbiology, **137**(12), 2857-65 (1991) and US Patent Nos. 5,576,298 and 5,837,685) Unlike the previously described antimycotics from *P. syringae* (e.g., syringomycins, syringotoxins and syringostatins), pseudomycins A-C contain hydroxyaspartic acid, aspartic acid, serine, dehydroaminobutyric acid, lysine and diaminobutyric acid. The peptide moiety for pseudomycins A, A', B, B', C, C' corresponds to L-Ser-D-Dab-L-Asp-L-Lys-L-Dab-L-aThr-Z-Dhb-L-Asp(3-OH)-L-Thr(4-Cl) with the terminal carboxyl group closing a macrocyclic ring on the OH group of the N-terminal Ser. The analogs are distinguished by the N-acyl side chain, i.e., pseudomycin A is N-acylated by 3,4-dihydroxytetradeconoyl, pseudomycin A' by

3,4-dihydroxypentadecanoyl, pseudomycin B by 3-hydroxytetradecanoyl, pseudomycin B' by 3-hydroxydodecanoyl, pseudomycin C by 3,4-dihydroxyhexadecanoyl and pseudomycin C' by 3-hydroxyhexadecanoyl. (see i.e., Ballio, A., et al., "Novel bioactive lipodepsipeptides from *Pseudomonas syringae*: the pseudomycins," FEBS Letters, **355**(1), 96-100, (1994) and Coiro, V.M., et al., "Solution conformation of the *Pseudomonas syringae* MSU 16H phytotoxic lipodepsipeptide Pseudomycin A determined by computer simulations using distance geometry and molecular dynamics from NMR data," Eur. J. Biochem., **257**(2), 449-456 (1998).)

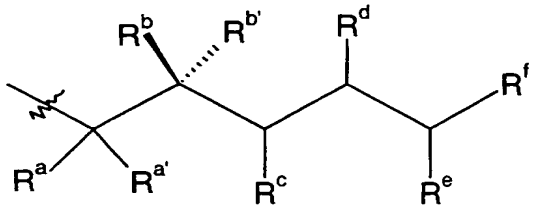
Pseudomycins are known to have certain adverse biological effects. For example, destruction of the endothelium of the vein, destruction of tissue, inflammation, and local toxicity to host tissues have been observed when pseudomycin is administered intraveneously. Therefore, there is a need to identify compounds within this class that are useful for treating fungal infections without the currently observed adverse side effects.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a pseudomycin prodrug represented by the following structure which is useful as an antifungal agent.



wherein R is



where

R^a and $R^{a'}$ are independently hydrogen or methyl, or either R^a or $R^{a'}$ is alkyl amino, taken together with R^b or $R^{b'}$ forms a six-membered cycloalkyl ring, a six-membered aromatic ring or a double bond, or taken together with R^c forms a six-membered aromatic ring;

R^b and $R^{b'}$ are independently hydrogen, halogen, or methyl, or either R^b or $R^{b'}$ is amino, alkylamino, α-acetoacetate, methoxy, or hydroxy;

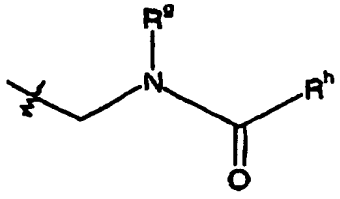
R^c is hydrogen, hydroxy, C_1-C_4 alkoxy, hydroxy C_1-C_4 alkoxy, or taken together with R^e forms a 6-membered aromatic ring or C_5-C_6 cycloalkyl ring;

R^d is hydrogen;

R^e is hydrogen, or taken together with R^f is a six-membered aromatic ring, C_5-C_{14} alkoxy substituted six-membered aromatic ring, or C_5-C_{14} alkyl substituted six-membered aromatic ring, and

R^f is C_8-C_{18} alkyl, or C_5-C_{11} alkoxy;

R is

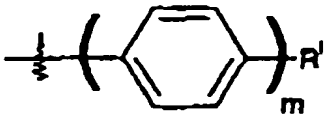


where

R^g is hydrogen, or C_1-C_{13} alkyl, and

R^h is C_1-C_{15} alkyl, C_4-C_{15} alkoxy, (C_1-C_{10} alkyl)phenyl, -$(CH_2)_n$-aryl, or -$(CH_2)_n$-(C_5-C_6 cycloalkyl), where n = 1 or 2; or

R is

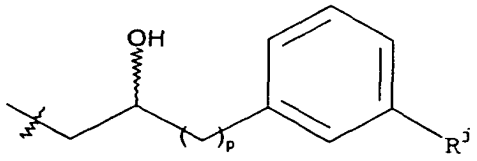


where

R^i is a hydrogen, halogen, or C_5-C_8 alk xy, and

m is 1, 2 or 3;

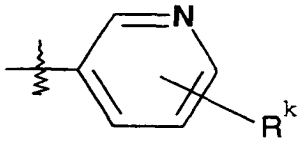
R is



where

R^j is C_5-C_{14} alkoxy or C_5-C_{14} alkyl, and p = 0, 1 or 2;

R is

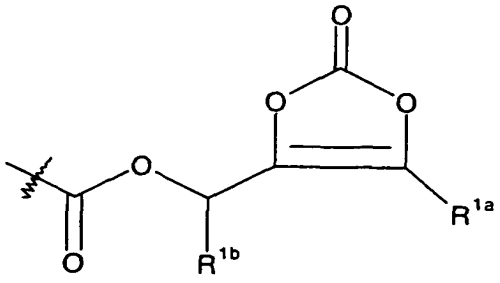


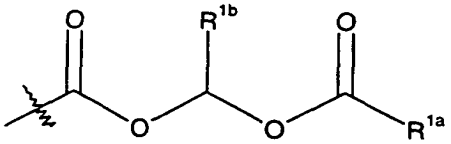
where

R^k is C_5-C_{14} alkoxy; or

R is $-(CH_2)-NR^m-(C_{13}$-C_{18} alkyl), where R^m is H, $-CH_3$ or $-C(O)CH_3$;

R^1 is independently hydrogen, an acyloxymethylene-1,3-dioxolen-2-one (e.g., compounds 1(a) depicted below), or an acyloxymethylenecarboxylate (e.g., compounds 1(b) depicted below)





1(a) 1(b)

where

R^{1a} is hydrogen, C_1-C_{10} alkyl, ~~C_1-C_{10}~~ C_2-C_{10} alkenyl, benzyl, or aryl and

R^{1b} is hydrogen or methyl

provided that at least one R^1 is an acyloxymethylene-1,3-dioxolen-2-one or an acyloxymethylenecarboxylate;

R^2 and R^3 are independently $-OR^{2a}$, or $-N(R^{2b})(R^{2c})$,

where

R^{2a} and R^{2b} are independently hydrogen, C_1-C_{10} alkyl (e.g., methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *i*-butyl, *s*-butyl, *t*-butyl, etc.), C_3-C_6 cycloalkyl (e.g., cyclopropyl, cyclobutyl, cyclopentyl, cyclopentylmethylene, methylcyclopentyl, cyclohexyl, etc.) hydroxy(C_1-C_{10})alkyl, alkoxy(C_1-C_{10})alkyl (e.g, methoxyethyl), or C_2-C_{10} alkenyl, amino(C_1-C_{10})alkyl, mono- or di-alkylamino(C_1-C_{10})alkyl, aryl(C_1-C_{10})alkyl (e.g., benzyl), heteroaryl(C_1-C_{10})alkyl (e.g., 3-pyridylmethyl, 4-pyridylmethyl), or cycloheteroalkyl(C_1-C_{10})alkyl (e.g., N-tetrahydro-1,4-oxazinylethyl and N-piperazinylethyl), or

R^{2b} is an alkyl carboxylate residue of an aminoacid alkyl ester (e.g., $-CH_2CO_2CH_3$, $-CH(CO_2CH_3)CH(CH_3)_2$, $-CH(CO_2CH_3)CH(phenyl)$, $-CH(CO_2CH_3)CH_2OH$, $-CH(CO_2CH_3)CH_2(p\text{-hydroxyphenyl})$,

$-CH(CO_2CH_3)CH_2SH$, $-CH(CO_2CH_3)CH_2(CH_2)_3NH_2$,

$-CH(CO_2CH_3)CH_2$(4- or 5-imidazole), $-CH(CO_2CH_3)CH_2CO_2CH_3$,

$-CH(CO_2CH_3)CH_2CO_2NH_2$, and the like), and

R^{2c} is hydrogen or C_1-C_6 alkyl; and

pharmaceutically acceptable salts and solvates thereof.

In another embodiment of the present invention, a pharmaceutical formulation is provided which includes the pseudomycin prodrug described above and a pharmaceutically acceptable carrier.

In yet another embodiment of the present invention, a method is provided for treating ~~an antifungal~~ fungal infection in an animal in need thereof, which comprises administering to the animal the pseudomycin prodrug described above. The use of the pseudomycin prodrug described above in the manufacture of a medicament for use in treating an antifungal infection in an animal is also provided.

Definitions

As used herein, the term "alkyl" refers to a hydrocarbon radical of the general formula C_nH_{2n+1} containing from 1 to 30 carbon atoms unless otherwise indicated. The alkane radical may be straight (e.g. methyl, ethyl, propyl, butyl, etc.), branched (e.g., isopropyl, isobutyl, tertiary butyl, neopentyl, etc.), cyclic (e.g., cyclopropyl, cyclobutyl, cyclopentyl, methylcyclopentyl, cyclohexyl, etc.), or multi-cyclic (e.g., bicyclo[2.2.1]heptane,

spiro[2.2]pentane, etc.). The alkane radical may be substituted or unsubstituted. Similarly, the alkyl portion of an alkoxy group, alkanoyl, or alkanoate have the same definition as above.

The term "alkenyl" refers to an acyclic hydrocarbon containing at least one carbon carbon double bond. The alkene radical may be straight, branched, cyclic, or multi-cyclic. The alkene radical may be substituted or unsubstituted. The alkenyl portion of an alkenoxy, alkenoyl or alkenoate group has the same definition as above.

The term "aryl" refers to aromatic moieties having single (e.g., phenyl) or fused ring systems (e.g., naphthalene, anthracene, phenanthrene, etc.). The aryl groups may be substituted or unsubstituted.

Within the field of organic chemistry and particularly within the field of organic biochemistry, it is widely understood that significant substitution of compounds is tolerated or even useful. In the present invention, for example, the term alkyl group allows for substitutents which is a classic alkyl, such as methyl, ethyl, propyl, hexyl, isooctyl, dodecyl, stearyl, etc. The term "group" specifically envisions and allows for substitutions on alkyls which are common in the art, such as hydroxy, halogen, alkoxy, carbonyl, keto, ester, carbamato, etc., as well as including the unsubstituted alkyl moiety. However,

it is generally understood by those skilled in the art that the substituents should be selected so as to not adversely affect the pharmacological characteristics of the compound or adversely interfere with the use of the medicament. Suitable substituents for any of the groups defined above include alkyl, alkenyl, alkynyl, aryl, halo, hydroxy, alkoxy, aryloxy, mercapto, alkylthio, arylthio, mono- and di-alkyl amino, quaternary ammonium salts, aminoalkoxy, hydroxyalkylamino, aminoalkylthio, carbamyl, carbonyl, carboxy, glycolyl, glycyl, hydrazino, guanyl, and combinations thereof.

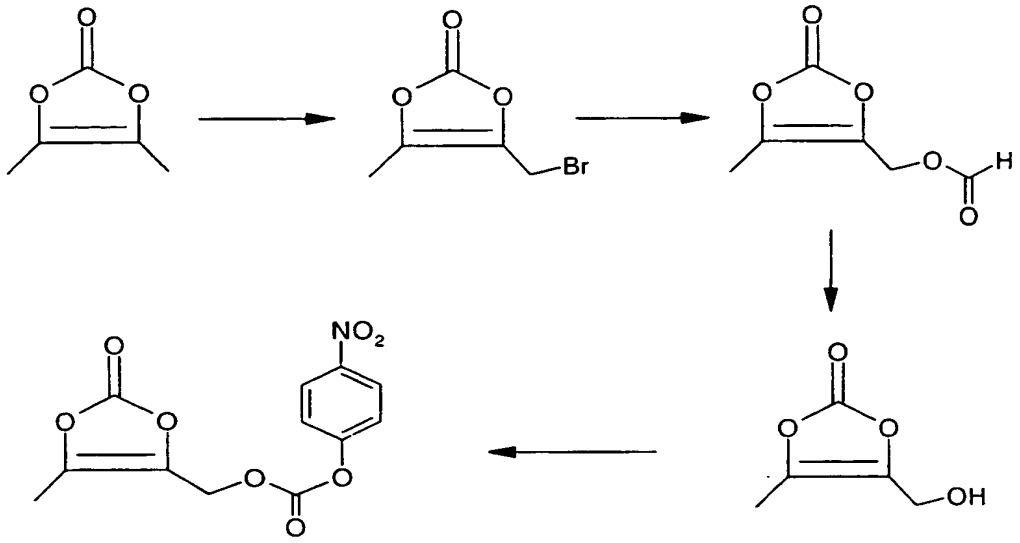
The term "prodrug" refers to a class of drugs which result in pharmacological action due to conversion by metabolic processes within the body (i.e., biotransformation). In the present invention, the pseudomycin prodrug compounds contain linkers that can be cleaved by esterases in the plasma to produce the active drug.

The term "animal" refers to humans, companion animals (e.g., dogs, cats and horses), food-source animals (e.g., cows, pigs, sheep and poultry), zoo animals, marine animals, birds and other similar animal species.

DETAILED DESCRIPTION OF THE INVENTION

Applicants have discovered that a prodrug derivative of the pseudomycin natural or semi-synthetic products provide less adverse side effects than the corresponding natural products and maintains *in vivo* efficacy against *C. albican*, *C. neoformans*, and *A. fumigatus*. The prodrug is produced by acylating at least one of the pendant amino groups attached to the lysine or 2,4-diaminobutyric acid peptide units in the pseudomycin cyclopeptide ring system to form an acyl substituent(s). The acylating agent (or linker) is generally a acyloxymethylene-1,3-dioxolen-2-one or acyloxymethylenecarboxylate acylating compound containing a suitable leaving group such that a carbamate linkage with the pendant amino group on the pseudomycin structure can be formed. Suitable leaving groups are well known to those skilled in the art and include groups such as *p*-nitrophenoxy and N-oxysuccinimide.

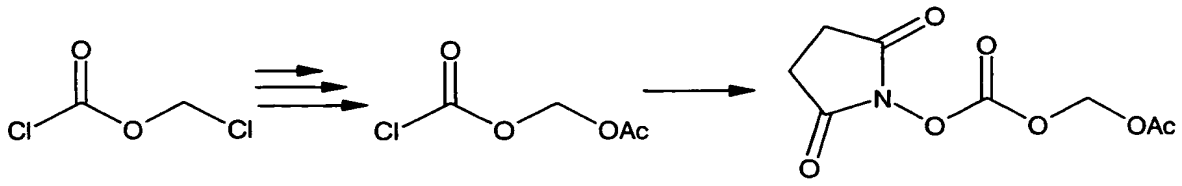
An acyloxymethylene-1,3-dioxolen-2-one acylating compound may be synthesized using the synthetic route shown in scheme I below. For illustrative purposes, a specific acylating compound is depicted. However, it will be understood by those skilled in the art that one could synthesize a variety of derivatives using the same basic synthetic method.



Scheme I

For a more detailed description of the synthetic procedures, see the preparation section of the Examples below.

The acyloxymethylenecarboxylate acylating compound may be synthesized using the synthetic route shown in scheme II below. For illustrative purposes, a specific acylating compound is depicted. However, it will be understood by those skilled in the art that one could synthesize a variety of derivatives using the same basic synthetic method.



Scheme II

For a more detailed description of the synthetic procedures, see the preparation section of the Examples below.

As discussed earlier, pseudomycins are natural products isolated from the bacterium *Pseudomonas syringae* that have been characterized as lipodepsinonapetpides containing a cyclic peptide portion closed by a lactone bond and including the unusual amino acids 4-chlorothreonine (ClThr), 3-hydroxyaspartic acid (HOAsp), 2,3-dehydro-2-aminobutyric acid (Dhb), and 2,4-diaminobutyric acid (Dab). Methods for growth of various strains of *P. syringae* to produce the different pseudomycin analogs (A, A', B, B', C, and C') are described below and described in more detail in PCT Patent Application Serial No. PCT/US00/08728 filed by Hilton, et al. on April 14, 2000 entitled "Pseudomycin Production by *Pseudomonas Syringae,*" incorporated herein by reference, PCT Patent Application Serial No. PCT/US00/08727 filed by Kulanthaivel, et al. on April 14, 2000 entitled "Pseudomycin Natural Products," incorporated herein by reference, and U.S. Patent Nos. 5,576,298 and 5,837,685, each of which are incorporated herein by reference.

Isolated strains of *P. syringae* that produce one or more pseudomycins are known in the art. Wild type strain MSU 174 and a mutant of this strain generated by transposon mutagenesis, MSU 16H are described in U.S. Patent Nos. 5,576,298 and 5,837,685; Harrison, et al., "Pseudomycins, a

family of novel peptides from *Pseudomonas syringae* possessing broad-spectrum antifungal activity," J. Gen. Microbiology, **137**, 2857-2865 (1991); and Lamb et al., "Transposon mutagenesis and tagging of fluorescent pseudomonas: Antimycotic production is necessary for control of Dutch elm disease," Proc. Natl. Acad. Sci. USA, **84**, 6447-6451 (1987).

A strain of P. syringae that is suitable for production of one or more pseudomycins can be isolated from environmental sources including plants (e.g., barley plants, citrus plants, and lilac plants) as well as, sources such as soil, water, air, and dust. A preferred stain is isolated from plants. Strains of P. syringae that are isolated from environmental sources can be referred to as wild type. As used herein, "wild type" refers to a dominant genotype which naturally occurs in the normal population of P. syringae (e.g., strains or isolates of P. syringae that are found in nature and not produced by laboratory manipulation). Like most organisms, the characteristics of the pseudomycin-producing cultures employed (P. syringae strains such as MSU 174, MSU 16H, MSU 206, 25-B1, 7H9-1) are subject to variation. Hence, progeny of these strains (e.g., recombinants, mutants and variants) may be obtained by methods known in the art.

P. syringae MSU 16H is publicly available from the American Type Culture Collection, Parklawn Drive, Rockville, MD, USA as Accession No. ATCC 67028. *P. syringae* strains 25-B1, 7H9-1, and 67 H1 were deposited with the American Type Culture Collection on March 23, 2000 and were assigned the following Accession Nos.:

25-B1	Accession No. PTA-1622
7H9-1	Accession No. PTA-1623
67 H1	Accession No. PTA-1621

Mutant strains of *P. syringae* are also suitable for production of one or more pseudomycins. As used herein, "mutant" refers to a sudden heritable change in the phenotype of a strain, which can be spontaneous or induced by known mutagenic agents, such as radiation (e.g., ultraviolet radiation or x-rays), chemical mutagens (e.g., ethyl methanesulfonate (EMS), diepoxyoctane, N-methyl-N-nitro-N'-nitrosoguanine (NTG), and nitrous acid), site-specific mutagenesis, and transposon mediated mutagenesis. Pseudomycin-producing mutants of *P. syringae* can be produced by treating the bacteria with an amount of a mutagenic agent effective to produce mutants that overproduce one or more pseudomycins, that produce one pseudomycin (e.g., pseudomycin B) in excess over other pseudomycins, or that produce one or more pseudomycins under advantageous growth conditions. While the type and amount of mutagenic agent to

be used can vary, a preferred method is to serially dilute NTG to levels ranging from 1 to 100 µg/ml. Preferred mutants are those that overproduce pseudomycin B and grow in minimal defined media.

Environmental isolates, mutant strains, and other desirable strains of *P. syringae* can be subjected to selection for desirable traits of growth habit, growth medium nutrient source, carbon source, growth conditions, amino acid requirements, and the like. Preferably, a pseudomycin producing strain of *P. syringae* is selected for growth on minimal defined medium such as N21 medium and/or for production of one or more pseudomycins at levels greater than about 10 µg/ml. Preferred strains exhibit the characteristic of producing one or more pseudomycins when grown on a medium including three or fewer amino acids and optionally, either a lipid, a potato product or combination thereof.

Recombinant strains can be developed by transforming the *P. syringae* strains, using procedures known in the art. Through the use of recombinant DNA technology, the *P. syringae* strains can be transformed to express a variety of gene products in addition to the antibiotics these strains produce. For example, one can modify the strains to

introduce multiple copies of the endogenous pseudomycin-biosynthesis genes to achieve greater pseudomycin yield.

To produce one or more pseudomycins from a wild type or mutant strain of P. syringae, the organism is cultured with agitation in an aqueous nutrient medium including an effective amount of three or fewer amino acids, preferably glutamic acid, glycine, histidine, or a combination thereof. Alternatively, glycine is combined with one or more of a potato product and a lipid. Culturing is conducted under conditions effective for growth of *P. syringae* and production of the desired pseudomycin or pseudomycins. Effective conditions include temperatures from about 22ºC to about 27ºC, and a duration of about 36 hours to about 96 hours. Controlling the concentration of oxygen in the medium during culturing of *P. syringae* is advantageous for production of a pseudomycin. Preferably, oxygen levels are maintained at about 5 to 50% saturation, more preferably about 30% saturation. Sparging with air, pure oxygen, or gas mixtures including oxygen can regulate the concentration of oxygen in the medium.

Controlling the pH of the medium during culturing *of P. syringae* is also advantageous. Pseudomycins are labile at basic pH, and significant degradation can occur if the pH of the culture medium is above about 6 for more than about 12 hours. Preferably, the pH of the culture medium is

maintained between 6 and 4. *P. syringae* can produce one or more pseudomycins when grown in batch culture. However, fed-bath or semi-continuous feed of glucose and optionally, an acid or base (e.g., ammonium hydroxide) to control pH, enhances production. Pseudomycin production can be further enhanced by using continuous culture methods in which glucose and ammonium hydroxide are fed automatically.

Choice of *P. syringae* strain can affect the amount and distribution of pseudomycin or pseudomycins produced. For example, strains MSU 16H and 67 H1 each produce predominantly pseudomycin A, but also produce pseudomycin B and C, typically in ratios of 4:2:1. Strain 67 H1 typically produces levels of pseudomycins about three to five fold larger than are produced by strain MSU 16H. Compared to strains MSU 16H and 67 H1, strain 25-B1 produces more pseudomycin B and less pseudomycin C. Strain 7H9-1 are distinctive in producing predominantly pseudomycin B and larger amount of pseudomycin B than other strains. For example, this strain can produce pseudomycin B in at least a ten fold excess over either pseudomycin A or C.

Alternatively, the prodrug can be formed from an N-acyl semi-synthetic compound. Semi-synthetic pseudomycin compounds may be synthesized by exchanging the N-acyl group on the L-serine unit. Examples of various N-acyl derivatives are described in PCT Patent Application Serial

No. _____, Belvo, et al., filed evendate herewith entitled "Pseudomycin N-Acyl Side-Chain Analogs" and incorporated herein by reference. In general, four synthetic steps are used to produce the semi-synthetic compounds from naturally occurring pseudomycin compounds: (1) selective amino protection; (2) chemical or enzymatic deacylation of the N-acyl side-chain; (3) reacylation with a different side-chain; and (4) deprotection of the amino groups.

The pendant amino groups at positions 2, 4 and 5 may be protected using any standard means known to those skilled in the art for amino protection. The exact genus and species of amino protecting group employed is not critical so long as the derivatized amino group is stable to the condition of subsequent reaction(s) on other positions of the intermediate molecule and the protecting group can be selectively removed at the appropriate point without disrupting the remainder of the molecule including any other amino protecting group(s). Suitable amino-protecting groups include benzyloxycarbonyl, *p*-nitrobenzyloxycarbonyl, *p*-bromobenzyloxycarbonyl, *p*-methoxybenxyloxycarbonyl, *p*-methoxyphenylazobenzyloxycarbonyl, *p*-phenylazobenzyloxycarbonyl, *t*-butyloxycarbonyl, cyclopentyloxycarbonyl, and phthalimido. Preferred amino protecting groups are *t*-butoxycarbonyl (*t*-Boc), allyloxycarbonyl (Alloc), phthalimido, and benzyloxycarbonyl

(CbZ or CBZ). Further examples of suitable protecting groups are described in T.W. Greene, "Protective Groups in Organic Synthesis," John Wiley and Sons, New York, N.Y., (2nd ed., 1991), at chapter 7.

The deacylation of a N-acyl group having a gamma or delta hydroxylated side chain (e.g., 3,4-dihydroxytetradeconoate) may be accomplished by treating the amino-protected pseudomycin compound with acid in an aqueous solvent. Suitable acids include acetic acid and trifluoroacetic acid. A preferred acid is trifluoroacetic acid. If trifluoroacetic acid is used, the reaction may be accomplished at or near room temperature. However, when acetic acid is used the reaction is generally ran at about 40°C. Suitable aqueous solvent systems include acetonitrile, water, and mixtures thereof. Organic solvents accelerate the reaction; however, the addition of an organic solvent may lead to other by-products. Pseudomycin compounds lacking a delta or gamma hydroxy group on the side chain (e.g., Pseudomycin B and C') may be deacylated enzymatically. Suitable deacylase enzymes include Polymyxin Acylase (164-16081 Fatty Acylase (crude) or 161-16091 Fatty Acylase (pure) available from Wako Pure Chemical Industries, Ltd.), or ECB deacylase. The enzymatic deacylation may be accomplished using standard deacylation procedures well known to those skilled in the art. For example, general

procedures for using polymyxin acylase may be found in Yasuda, N., et al, *Agric. Biol. Chem.*, 53, 3245 (1989) and Kimura, Y., et al., *Agric. Biol. Chem.*, 53, 497 (1989).

The deacylated product (also known as the pseudomycin nucleus) is reacylated using the corresponding acid of the desired acyl group in the presence of a carbonyl activating agent. "Carbonyl activating group" refers to a substituent of a carbonyl that promotes nucleophilic addition reactions at that carbonyl. Suitable activating substituents are those which have a net electron withdrawing effect on the carbonyl. Such groups include, but are not limited to, alkoxy, aryloxy, nitrogen containing aromatic heterocycles, or amino groups (e.g., oxybenzotriazole, imidazolyl, nitrophenoxy, pentachlorophenoxy, N-oxysuccinimide, N,N'-dicyclohexylisoure-O-yl, and N-hydroxy-N-methoxyamino); acetates; formates; sulfonates (e.g., methanesulfonate, ethanesulfonate, benzenesulfonate, and *p*-tolylsulfonate); and halides (e.g., chloride, bromide, and iodide).

A variety of acids may be used in the acylation process. Suitable acids include aliphatic acids containing one or more pendant aryl, alkyl, amino(including primary, secondary and tertiary amines), hydroxy, alkoxy, and amido groups; aliphatic acids containing nitrogen or oxygen within the aliphatic chain; aromatic acids substituted with alkyl, hydroxy, alkoxy and/or alkyl amino groups; and

heteroaromatic acids substituted with alkyl, hydroxy, alkoxy and/or alkyl amino groups.

Alternatively, a solid phase synthesis may be used where a hydroxybenzotriazole-resin (HOBt-resin) serves as the coupling agent for the acylation reaction.

Once the amino group is deacylated and reacylated (described above), then the amino protecting groups (at positions 2, 4 and 5) can be removed by hydrogenation in the presence of a hydrogenation catalyst (e.g., 10% Pd/C). When the amino protecting group is allyloxycarbonyl, then the protecting group can be removed using tributyltinhydride and triphenylphosphine palladium dichloride. This particular protection/deprotection scheme has the advantage of reducing the potential for hydrogenating the vinyl group of the Z-Dhb unit of the pseudomycin structure.

The prodrug is then produced by acylating at least one of the pendant amino groups attached to the lysine or 2,4-diaminobutyric acid peptide units of the N-acyl modified semi-synthetic pseudomycin compound to form the desired carbamate linkage.

Other modified prodrug pseudomycin compounds may be synthesized by amidation or esterification of the pendant carboxylic acid group of the aspartic acid and/or hydroxyaspartic acid units of the pseudomycin ring. Examples of various acid-modified derivatives are described

in PCT Patent Application Serial No. PCT/US00/15021, Chen, et al., filed evendate herewith entitled "Pseudomycin Amide & Ester Analogs" and incorporated herein by reference. The acid-modified derivatives may be formed by condensing any of the previously described prodrugs with the appropriate alcohol or amine to produce the respective ester or amide.

Formation of the ester groups may be accomplished using standard esterification procedures well-known to those skilled in the art. Esterification under acidic conditions typically includes dissolving or suspending the pseudomycin compound in the appropriate alcohol in the presence of a protic acid (e.g., HCl, TFA, etc.). Under basic conditions, the pseudomycin compound is generally reacted with the appropriate alkyl halide in the presence of a weak base (e.g., sodium bicarbonate and potassium carbonate).

Formation of the amide groups may be accomplished using standard amidation procedures well-known to those skilled in the art. However, the choice of coupling agents provides selective modification of the acid groups. For example, the use of benzotriazol-1-yloxy-tripyrrolidinophosphonium hexafluorophosphate(PyBOP) as the coupling agent allows one to isolate pure mono-amides at residue 8 and (in some cases) pure bis amides simultaneously. Whereas, the use of *o*-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium

tetrafluoroborate (TBTU) as the coupling agent favors formation of monoamides at residue 3.

The pseudomycin prodrug may be isolated and used per se or in the form of its pharmaceutically acceptable salt or solvate. The prodrug is prepared by forming at least one acyloxyalkylcarbamate linkage as described earlier. The term "pharmaceutically acceptable salt" refers to non-toxic acid addition salts derived from inorganic and organic acids. Suitable salt derivatives include halides, thiocyanates, sulfates, bisulfates, sulfites, bisulfites, arylsulfonates, alkylsulfates, phosphonates, monohydrogen-phosphates, dihydrogenphosphates, metaphosphates, pyrophosphonates, alkanoates, cycloalkylalkanoates, arylalkonates, adipates, alginates, aspartates, benzoates, fumarates, glucoheptanoates, glycerophosphates, lactates, maleates, nicotinates, oxalates, palmitates, pectinates, picrates, pivalates, succinates, tartarates, citrates, camphorates, camphorsulfonates, digluconates, trifluoroacetates, and the like.

The term "solvate" refers to an aggregate that comprises one or more molecules of the solute (i.e., pseudomycin prodrug compound) with one or more molecules of a pharmaceutical solvent, such as water, ethanol, and the like. When the solvent is water, then the aggregate is referred to as a hydrate. Solvates are generally formed by

dissolving the prodrug in the appropriate solvent with heat and slowing cooling to generate an amorphous or crystalline solvate form.

Each pseudomycin, semi-synthetic pseudomycin, pseudomycin prodrug and mixtures can be detected, determined, isolated, and/or purified by any variety of methods known to those skilled in the art. For example, the level of pseudomycin or pseudomycin prodrug activity in a broth or in an isolate or purified composition can be determined by antifungal action against a fungus such as Candida and can be isolated and purified by high performance liquid chromatography.

The active ingredient (i.e., pseudomycin prodrug) is typically formulated into pharmaceutical dosage forms to provide an easily controllable dosage of the drug and to give the patient, physician or veterinarian an elegant and easily handleable product. Formulations may comprise from 0.1% to 99.9% by weight of active ingredient, more generally from about 10% to about 30% by weight.

As used herein, the term "unit dose" or "unit dosage" refers to physically discrete units that contain a predetermined quantity of active ingredient calculated to produce a desired therapeutic effect. When a unit dose is administered orally or parenterally, it is typically provided in the form of a tablet, capsule, pill, powder

packet, topical composition, suppository, wafer, measured units in ampoules or in multidose containers, etc. Alternatively, a unit dose may be administered in the form of a dry or liquid aerosol which may be inhaled or sprayed.

The dosage to be administered may vary depending upon the physical characteristics of the animal, the severity of the animal's symptoms, the means used to administer the drug and the animal species. The specific dose for a given animal is usually set by the judgment of the attending physician or veterinarian.

Suitable carriers, diluents and excipients are well known to those skilled in the art and include materials such as carbohydrates, waxes, water soluble and/or swellable polymers, hydrophilic or hydrophobic materials, gelatin, oils, solvents, water, and the like. The particular carrier, diluent or excipient used will depend upon the means and purpose for which the active ingredient is being applied. The formulations may also include wetting agents, lubricating agents, surfactants, buffers, tonicity agents, bulking agents, stabilizers, emulsifiers, suspending agents, preservatives, sweeteners, perfuming agents, flavoring agents and combinations thereof.

A pharmaceutical composition may be administered using a variety of methods. Suitable methods include topical (e.g., ointments or sprays), oral, injection and inhalation.

The particular treatment method used will depend upon the type of infection being addressed.

In parenteral iv applications, the formulations are typically diluted or reconstituted (if freeze-dried) and further diluted if necessary, prior to administration. An example of reconstitution instructions for the freeze-dried product are to add ten ml of water for injection (WFI)to the vial and gently agitate to dissolve. Typical reconstitution times are less than one minute. The resulting solution is then further diluted in an infusion solution such as dextrose 5% in water (D5W), prior to administration.

Pseudomycin compounds have been shown to exhibit antifungal activity such as growth inhibition of various infectious fungi including Candida spp. (i.e., *C. albicans, C. parapsilosis, C. krusei, C. glabrata, C. tropicalis,* or *C. lusitaniaw*); Torulopus spp.(i.e., *T. glabrata*); Aspergillus spp. (i.e., *A. fumigatus*); Histoplasma spp. (i.e., *H. capsulatum*); Cryptococcus spp. (i.e., *C. neoformans*); Blastomyces spp. (i.e., *B. dermatitidis*); Fusarium spp.; Trichophyton spp., *Pseudallescheria boydii, Coccidioides immits, Sporothrix schenckii,* etc.

Consequently, the compounds and formulations of the present invention are useful in the preparation of medicaments for use in combating either systemic fungal infections or fungal skin infections. Accordingly, a method

is provided for inhibiting fungal activity comprising contacting the pseudomycin prodrug of the present invention with a fungus. A preferred method includes inhibiting *Candida albicans* or *Aspergillus fumigatus* activity. The term "contacting" includes a union or junction, or apparent touching or mutual tangency of a compound of the invention with a fungus. The term does not imply any further limitations to the process, such as by mechanism of inhibition. The methods are defined to encompass the inhibition of parasitic and fungal activity by the action of the compounds and their inherent antifungal properties.

A method for treating a fungal infection which comprises administering an effective amount of a pharmaceutical formulation of the present invention to a host in need of such treatment is also provided. A preferred method includes treating a *Candida albicans, Cryptococcus neoformans,* or *Aspergillus fumigatus* infection. The term "effective amount" refers to an amount of active compound which is capable of inhibiting fungal activity. The dose administered will vary depending on such factors as the nature and severity of the infection, the age and general health of the host, the tolerance of the host to the antifungal agent and the species of the host. The particular dose regimen likewise may vary according to these factors. The medicament may be given in a single daily dose

or in multiple doses during the day. The regimen may last from about 2-3 days to about 2-3 weeks or longer. A typical daily dose (administered in single or divided doses) contains a dosage level between about 0.01 mg/kg to 100 mg/kg of body weight of an active compound. Preferred daily doses are generally between about 0.1 mg/kg to 60 mg/kg and more preferably between about 2.5 mg/kg to 40 mg/kg. The host is generally an animal including humans, companion animals (e.g., dogs, cats and horses), food-source animals (e.g., cows, pigs, sheep and poultry), zoo animals, marine animals, birds and other similar animal species.

EXAMPLES

The following abbreviations are used through out the examples to represent the respective listed materials:

ACN - acetonitrile

TFA - trifluoroacetic acid

DMF - dimethylformamide

EDCI - 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride

BOC = t-butoxycarbonyl, $(CH_3)_3C$-O-C(O)-

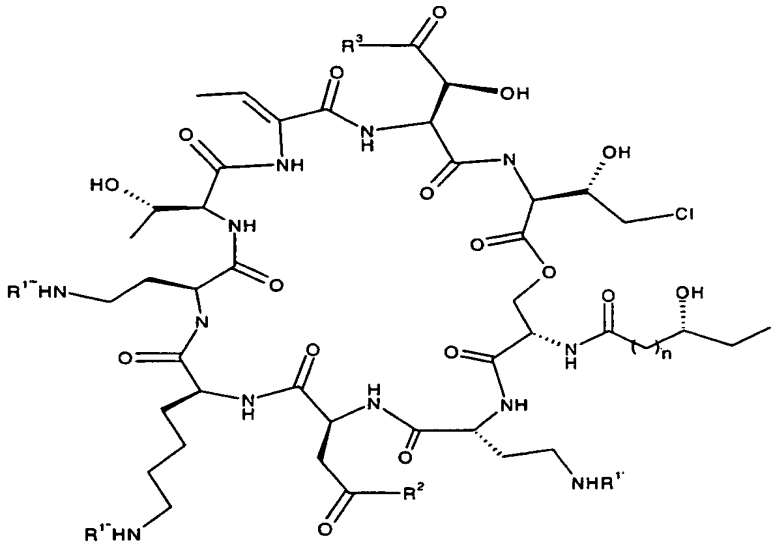
CBZ = benzyloxycarbonyl, $C_6H_5CH_2$-O-C(O)-

PyBOP = benzotriazol-1-yloxy-tripyrrolidinophosphonium hexafluorophosphate

TBTU = *o*-Benzotriazol-1-yl-N,N,N',N'-tetramethyluronium tetrafluoroborate

DIEA = N,N-diisopropylethylamine

The following structure II will be used to describe the products observed in Examples 1 through 7.



II

Detection and Quantification of Antifungal Activity:

Antifungal activity was determined *in vitro* by obtaining the minimum inhibitory concentration (MIC) of the compound using a standard agar dilution test or a disc-diffusion test. A typical fungus employed in testing antifungal activity is *Candida albicans*. Antifungal activity is considered significant when the test sample (50 µl) causes 10-12 mm diameter zones of inhibition on *C. albicans* x657 seeded agar plates.

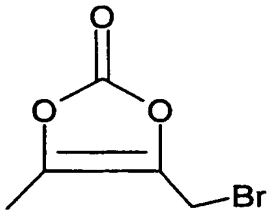
Tail Vein Toxicity:

Mice were treated intravenously (IV) through the lateral tail vein with 0.1 ml of testing compound (20 mg/kg) at 0, 24, 48 and 72 hours. Two mice were included in each group. Compounds were formulated in 5.0% dextrose and sterile water for injection. The mice were monitored for 7 days following the first treatment and observed closely for signs of irritation including erythema, swelling, discoloration, necrosis, tail loss and any other signs of adverse effects indicating toxicity.

The mice used in the study were outbred, male ICR mice having an average weight between 18-20 g (available from Harlan Sprangue Dawley, Indianapolis, IN).

Preparations

Preparation of 4-Bromomethyl-5-methyl-1,3-dioxolene-2-one (1a-1):

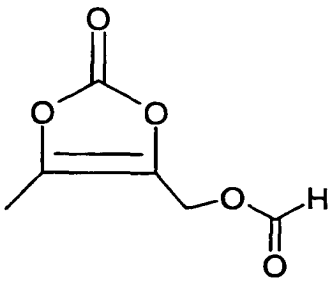


1a-1

A mixture of 0.1 mole of 4,5-Dimethyl-1,3-dioxolene-2-one, 0.1 mole of N-bromosuccinimide and 0.1 g of 2,2-

azobis(2-methylpropionitrile) in 70 ml of carbontetrachloride (CCl_4) was heated to reflux. After six hours, the mixture was cooled down with ice and filtered. The filtrate was washed with 2x50 ml of water, 2x50 ml of a sodium chloride solution and an additional 50 ml of water. The solution was dried over sodium sulfate and evaporated to dryness, and dried under vacuum to yield 16.5 g (85% yield) of an oil having 1H-NMR data consistent with structure 1a-1.

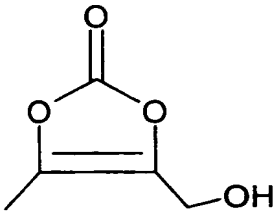
Preparation of Compound (1a-2):



1a-2

Compound 1a-2 was synthesized using the procedures described in Synthetic Communication, **22**(9), 1297 (1992) to yield 11.5 g (78% yield) of a crude oil.

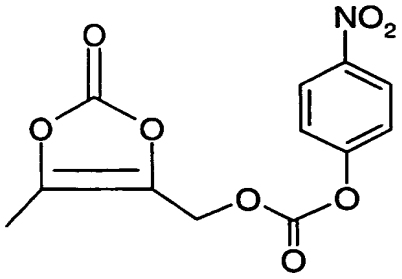
Preparation of the Compound 1a-3:



1a-3

A mixture of 11.5 g of Compound 1a-2 (crude oil), 500 ml of 37% HCl and 300 ml of methanol was allowed to stir overnight at 4ºC. The mixture was then concentrated to form an oil. Purification by column chromatography (1:1 ethylacetate/hexane) yielded 5.27 g (33.8%) of product having 1H-NMR data consistent with structure 1a-3.

Preparation of Compound 1a-4:

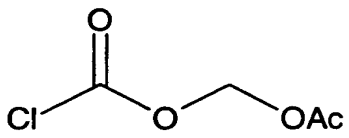


1a-4

A mixture of 3.0 g of Compound 1a-3 and 2.02 g of pyridine in 30 ml of chloroform was cooled to 0-4ºC. A solution of 5.08 g of p-nitrophenyl-chloroformate in 30 ml

of chloroform was added to the mixture and allowed to stir for about 4.5 hours. The mixture was washed with cooled 1% sodium hydroxide (3x30 ml), 1N HCl (2x30 ml), water (2x30 ml) and brine (2x30 ml). The solution was dried over sodium sulfate, filtered, and washed with dichloromethane. Removal of the solvent yielded an oil which solidified upon standing. The solid was picked up in 10 ml of dichloromethane and hexane was added to form a precipitate. The mixture was filtered, washed with hexane, and dried under vacuum overnight to yield 6.39 g (94% yield) product having 1H-NMR data consistent with structure 1a-4.

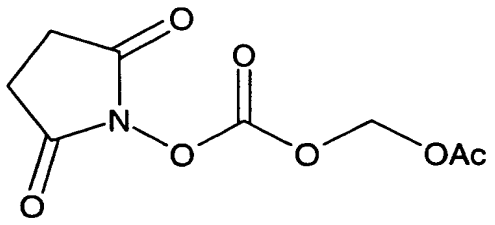
Preparation of Compound 1b-1:



1b-1

Compound **1b-1** can be synthesized using the procedures described in Synthesis, 1159 (1990).

Preparation of Compound 1b-2:



1b-2

A solution of 4.57 g (30 mmol) crude 1b-1 in 40 ml of dichloromethane was added at 0ºC to a solution of 3.91 g (34 mmol) N-hydroxy succinimide and 2.7 g (34 mmol) pyridine in 100 ml of dichloromethane. After stirring at 0ºC for 30 minutes, the mixture was allowed to stand at room temperature overnight. The mixture was then washed with water four times and the organic phase was dried over sodium sulfate. Upon filtration, the solvent was evaporated to give 4.0 g (58% yield) of an oily crude product having 1H-NMR data consistent with structure 1b-2.

Preparation of CBZ-Protected Pseudomycin B (2a-1):

Dissolve/suspend pseudomycin B in DMF (20 mg/ml, Aldrich Sure Seal). While stirring at room temperature add *N*-(Benzyloxycarbonyloxy)succinimide (6 eq). Allow to stir at room temperature for 32 hours. Monitor reaction by HPLC

(4.6x50 mm, 3.5 µm, 300-SB, C8, Zorbax column). Concentrate reaction to 10 ml on a high vacuum roto-evaporator at room temperature. Put material in freezer until ready to prep by chromatography. Reverse phase preparative HPLC yields an amorphous, white solid (Compound **2a-1**) after lyophilization.

Preparation of Compound 2b-1:

$R^{1'}$, $R^{1''}$ and $R^{1'''}$ = H
R^2 = -NH(cyclopropyl)
R^3 = -OH

2b-1

CBZ-protected pseudomycin B (2a-1)(400 mg, 0.25 mmol) is dissolved in 4 ml dry DMF. TBTU (79 mg, 0.25 mmol), DIEA (200 µl, 6 equivalents) and cyclopropylamine (14.2 mg, 0.25 mmol) were added sequentially. The reaction was stirred at room temperature under nitrogen while being monitored by HPLC. Upon completion the reaction was concentrated *in vacuo*. The crude product purified by preparative HPLC. Lyophilization yielded 209.2 mg (51.1%) of a colorless powder.

The 3-amido compound (279.1 mg, 0.169 mmol) was hydrogenated under hydrogen balloon catalyzed by 10% Pd/C in 1% HOAc/MeOH for 45 minutes. The reaction was filtered and concentrated *in vacuo*. The residue was picked up in a 1:1 mixture of water:ACN and then lyophilized to give 208.3 mg

(98.6%) of a colorless powder (**2b-1**). The structure was verified by H^1-NMR.

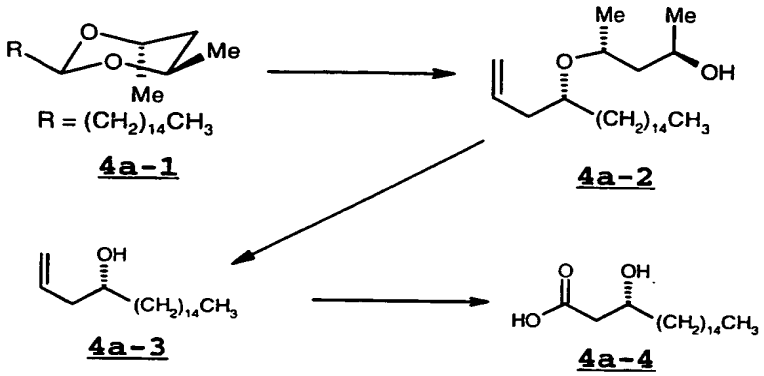
Preparation of Compound 3a-1:

$R^{1'}$, $R^{1''}$ and $R^{1'''}$ = H
R^2 = $-OCH_3$
R^3 = $-OCH_3$

3a-1

A 50 ml round bottom flask was charged with 10 ml of absolute ethanol and 251.7 mg of CBZ-protected pseudomycin B (2a-1) (0.156 mmol). To this mixture was added ~ 1 ml of acidified ethanol (previously acidified using HCl gas) and the reaction was allowed to stir at room temperature overnight. The solvent was then removed *in vacuo* and the residue was carried on to the next step without further purification by dissolving it in a solution of 10 ml MeOH/1.5 ml glacial AcOH. Standard hydrogenolysis using 249.7 mg of 10% Pd/C for 30 minutes, removal of the catalyst via filtration and purification via preparatory HPLC yielded 120.9 mg of Compound 3a-1 after lyophilization. MS (Ionspray) calcd for $C_{55}H_{96}ClN_{12}O_{19}$ $(M+H)^+$ 1264.89, found 1264.3.

Preparation of C-18 side-chain (4a-1):



4a-1 **4a-2** **4a-3** **4a-4**

To a dichloromethane solution (190 mL) of the chiral acetal **4a-1** (6.22 g, 19.1 mmol) was added at -78°C trimethylallylsilane (10.9 mL, 68.69 mmol), followed by neat $TiCl_4$ (2.94 mL, 26.71 mmol). The reaction was stirred at -78°C for 1 hr and then at -40°C for 2 hr. At this point, the reaction was quenched with methanol (15 mL) and diluted with dichloromethane (200 mL). The resulting reaction mixture was washed with 1N HCl (2 x 50 mL), water and brine. The organic layer was dried and conc. in vacuo to give a residue, which was purified by silica gel chromatography (10% EtOAc/Hexanes) to give 5.51 g (78%) of the desired product **4b-1**.

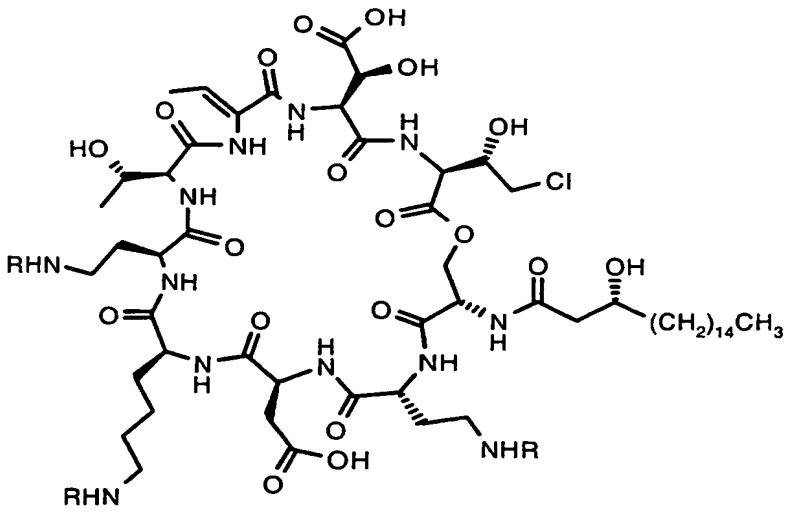
To a dichloromethane solution (155 mL) of **4b-1** (8.56 g, 23.3 mmol) was added PCC (10.0 g, 46.5 mmol). The reaction was stirred at rt for 18 hr, and then filtered through a pad

of Celite. The filtrates were concentrated in vacuo to give a reddish residue, which was purified by silica gel chromatography (10% EtOAc/Hexanes) to give 8.36 g (80%) of the methyl ketone intermediate (structure not shown). The intermediate obtained herein (8.36 g, 22.8 mmol) was dissolved in THF (60 mL) and MeOH (30 mL). To this solution was added 7.5 M KOH (15 mL). After stirring 3 hr at rt, the solvent was partially removed. The remaining reaction mixtures were diluted with EtOAc/Et2O (3:1 ratio, 350 mL). The organic layer was washed with water (3 x 50 mL) and brine. The resulting organic layer was dried and conc. in vacuo to give a residue, which was purified by silica gel chromatography (10% EtOAc/Hexanes) to afford 6.22 g (96&) of the desired product **4c-1** as white solids.

Carbinol **4c-1** (6.22 g, 22.0 mmol) was dissolved in an aqueous THF solution (5.5 mL water and 55 mL THF). To this solution was added NMO (4.42 g, 33.0 mmol), followed by OsO_4 (280 mg dissolved in THF, 1.10 mmol). The reaction stirred at rt overnight. At this time, sodium bisulfide (4 g) was added. The reaction was stirred for 2 hr, and then diluted with EtOAc (300 mL). The whole mixture was washed with water (2 x 40 mL) and brine. The resulting organic layer was dried and conc. *in vacuo* to give the corresponding triol intermediate. This material was dissolved in MeOH (200 mL) and water (40 mL). To this solution was added $NaIO_4$ (10.6 g,

49.5 mmol). After stirring at rt for 1hr, the reaction was filtered through Celite and purified by short column silica gel chromatography (30% EtOAc/Hexanes) to afford ~10 g (>100%) crude beta-hydroxyl aldehyde. The impuried aldehyde thus obtain was dissolved in t-BuOH (100 mL) and cyclohexene (14 mL). To this solution at rt was added an aqueous solution (50 mL) of $NaClO_2$ (15.97 g, 176 mmol) and KH_2PO_4 (17.8 g, 132 mmol). The reaction was stirred at rt for 6 hr and then quenched at 0°C with 5N HCl to pH=4. The reaction was extracted with 3:1 mix-solvent EtOAc/Et_2O (3 x 250 mL). The organic layer was washed with brine and dried and conc. to provide 7.3 g (>100%) of the crude acid **4d-1**, which was used directly for the coupling reaction.

Preparation of Pseudomycin C-18 (Compound 4b-2):



4a-2 R = Cbz
4b-2 R = H

The crude acid **4d-1** (2.1 g, 6.99 mmol) was dissolved in dry THF (20 mL) and DMF (20 mL). To this solution was added HOBt (1.23 g, 9.08 mmol) and EDCI (1.74 g, 9.08 mmol). After stirring at rt for 8 hr, CBZ-protected pseudomycin nucleus (3.87 g, 2.80 mmol) was added. The reaction was stirred at rt for 2 days. At this point, the solvent was partially removed. The reaction mixture was loaded onto preparative reverse phase HPLC system for purification (4 injections). Upon lyophilization, 550 mg (12%) of CBZ-protected C18 acyl derivative **4a-2** along with HOBt activated side chain ester (1 g) was isolated. The recovered side chain ester (1.0 g, 2.40 mmol) was next reacted with CBZ-protected pseudomycin nucleus (1.33 g, 0.96 mmol) in dry THF and DMF (10 mL each). Following the same purification procedure just mentioned, additional amounts of the CBZ-protected C18 derivative **4a-2** (606 mg, 38%) were obtained.

To a 10% HOAc/MeOH solution (55 mL) of CBZ-protected C18 derivative **4a-2** (550 mg, 0.33 mmol) was added at -78°C Pd/C (550 mg, 10% palladium content). The reaction was subjected to hydrogenation under 1.5 atm. pressure for 40 mim. The progress of the reaction was monitored by analytic HPLC. Upon completion, the catalyst was filtered and the filtrates were conc. in vacuo at 30°C. The resulting residue

was redissolved in 1:1 aqueous acetonitrile and lyophilized to give 250 mg (60%) of the desired product **4b-2**.

In each of the following Examples a specific pseudomycin compound is used as the starting material; however, those skilled in art in the art will recognize that other N-acyl derivatives may be synthesized using the same procedures except starting with a pseudomycin compound having a different N-acyl group.

Example 1

The following example demonstrates the formation of mono-, di- and tri-substituted acyloxyalkylcarbamate prodrugs of pseudomycin C' (n = 12, R^2 and R^3 = -OH).

To a DMF solution (1 liter) containing Pseudomycin C' (1.5 g, 1 eq.) was added 1.5 eq. of Compound 1a-4 and the mixture was stirred at room temperature for approximately 3 days. The solvent was partially removed and the residue purified by reverse-phase HPLC (Waters™, Delta Pak C18 column) to yield the following products and mixture of products:

86 mg of pure mono substituted pseudomycin C'(**1-1**);

87 mg of a mixture of mono-substituted pseudomycin C'(**1-2**);

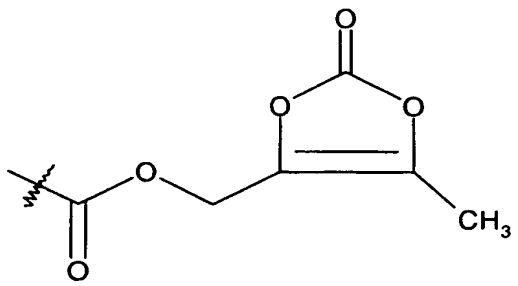
177 mg of a mixture of di-substituted pseudomycin C'(**1-3**);

132 mg of pure di-substituted pseudomycin C'(**1-4**); and

248 mg of tri-substituted pseudomycin C'(**1-5**).

No irritation of the tail vein was observed for the tri-substituted prodrug or the mixture of di-substituted prodrugs. Some irritation was observed with the pure mono-substituted, mixture of mono-substituted and pure di-substituted prodrug samples. In comparison, unsubstituted pseudomycin C' and unsubstituted pseudomycin B clearly showed irritation of the tail vein. All of the samples indicated significant *in vivo* efficacy except the pure di-substituted prodrug sample (ED_{50} > 20 mg/kgx4).

Samples of mono-, di- and tri-substituted prodrugs of structure II above where $R^{1'}$, $R^{1''}$ and/or $R^{1'''}$ is



and n is equal to 10, 12 and 14 have also been made using the same procedure described above.

Compounds 1-1 and 1-5 exhibited similar *in vivo* efficacy as the parent compound pseudomycin C'. No evidence of tail vein irritation was observed for Compound 1-5 and an improved tail vein toxicity profile was observed for Compound 1-1. Surprisingly, no *in vivo* efficacy was observed for Compound 1-4.

Example 2

The following examples illustrates the formation of mono-, di- and tri-substituted acyloxyalkylcarbamate prodrugs of pseudomycin B (n = 10, R^2 and R^3 = -OH).

To a solution of Pseudomycin B (2.0 g, 1.65 mmol) dissolved in 500 ml of dimethylformamide was added 574 mg (2.47 mmol) Compound 1b-2. The mixture was stirred at room temperature overnight. The solution was then concentrated to about 50 ml and the products purified by HPLC using a gradient elution scheme: 0-30% 0.1% TFA/ACN in 5 minutes and 30-70% TFA/ACN in 40 minutes. A combined yield of 59% was observed.

Three of the isolated products (mono-substituted prodrug (Compound **2-1**) where $R^{1'}$ and $R^{1''}$ = H and $R^{1'''}$ = $-C(O)OCH_2OAc$, the di-substituted prodrug (Compound **2-2**) where $R^{1'}$ and $R^{1''}$ = $-C(O)OCH_2OAc$ and $R^{1'''}$ = H and the tri-substituted prodrug (Compound **2-3**) where $R^{1'}$, $R^{1''}$ and $R^{1'''}$ = $-C(O)OCH_2OAc$) were all tested and demonstrated *in vivo* efficacy against murine systemic Candidiasis. However, the tail vein toxicity was positive.

Example 3

Using the same general procedures described above in Example 2, mono-, di- and tri-substituted prodrugs of Pseudomycin B (n = 10, R^2 and R^3 = -OH)) were prepared where

$R^{1'}$, $R^{1''}$ and/or $R^{1'''}$ = $-C(O)OCH_2OC(O)C(CH_3)_3$. The following five samples were isolated:

3-1 mono-substituted $R^{1'''}$

3-2 mixed mono-substituted $R^{1'}$ and $R^{1''}$

3-3 mixed di-substituted $R^{1'''}$ + $R^{1'}$ and $R^{1'''}$ + $R^{1''}$

3-4 di-substituted $R^{1'}$ + $R^{1''}$

3-5 trisubstituted $R^{1'}$ + $R^{1''}$ + $R^{1'''}$

Samples 3-1, 3-3 and 3-5 each demonstrated negative tail vein toxicity. All five samples demonstrated *in vivo* efficacy against murine systemic Candidiasis.

Example 4

Using the same general procedures described above in Example 2, mono-, di- and tri-substituted prodrugs of Pseudomycin C' (n = 12, R^2 and R^3 = -OH) were prepared where $R^{1'}$, $R^{1''}$ and/or $R^{1'''}$ = $-C(O)OCH_2OC(O)C(CH_3)_3$. The following five samples were isolated:

4-1 mono-substituted $R^{1'''}$

4-2 mixed mono-substituted $R^{1'}$ and $R^{1''}$

4-3 mixed di-substituted $R^{1'''}$ + $R^{1'}$ and $R^{1'''}$ + $R^{1''}$

4-4 di-substituted $R^{1'}$ + $R^{1''}$

4-5 trisubstituted $R^{1'}$ + $R^{1''}$ + $R^{1'''}$

Sample 4-1 was not tested. Samples 4-3, 4-4 and 4-5 all demonstrated negative tail vein toxicity. Samples 4-2, 4-3,

4-4 and 4-5 all demonstrated *in vivo* efficacy against murine systemic Candidiasis.

Example 5

Using the same general procedures described above in Example 2, mono-, di- and tri-substituted prodrugs of Pseudomycin B (n = 10) were prepared where $R^{1'}$, $R^{1''}$ and/or $R^{1'''}$ = $-C(O)OCH(CH_3)OC(O)CH_3$. Only the trisubstituted derivative (Compound **5-1**) was tested. The trisubstituted compound demonstrated negative tail vein toxicity.

Example 6

Using the same general procedures described above in Example 2, mono-, di- and tri-substituted prodrugs of Pseudomycin B (n = 10, R^2 and R^3 = -OH) were prepared where $R^{1'}$, $R^{1''}$ and/or $R^{1'''}$ = $-C(O)OCH_2OC(O)CH_2CH_3$. Only the trisubstituted derivative (Compound **6-1**) was tested. The trisubstituted compound demonstrated good *in vivo* efficacy against murine systemic Candidiasis without tail vein irritation.

Example 7

Using the same general procedures described above in Example 2, mono-, di- and tri-substituted prodrugs of Pseudomycin B (n = 10, R^2 and R^3 = -OH) were prepared where $R^{1'}$, $R^{1''}$ and/or $R^{1'''}$ = $-C(O)OCH_2OC(O)CH(CH_3)CH_3$. Only the

tri-substituted derivative (Compound **7-1**) was tested. The trisubstituted compound demonstrated good *in vivo* efficacy against murine systemic Candidiasis without tail vein irritation.

Example 8

Examples 8 and 9 illustrate the synthesis of prodrugs from semi-synthetic pseudomycin compounds where the pendant N-acyl group of the L-serine unit of the pseudomycin structure has been modified.

Using the same general procedures described above in Example 2, mono-, di- and tri-substituted prodrugs of Pseudomycin C-18 (n = 14, R^2 and R^3 = -OH)(**4b-2**) were prepared where $R^{1'}$, $R^{1''}$ and/or $R^{1'''}$ = $-C(O)OCH_2OC(O)C(CH_3)_3$. Only the trisubstituted derivative (Compound **8-1**) was tested. The trisubstituted compound demonstrated negative tail vein toxicity.

Example 9

Using the same general procedures described above in Example 2, mono-, di- and tri-substituted prodrugs of Pseudomycin C-18 (n = 14, R^2 and R^3 = -OH)(**4b-2**) were prepared where $R^{1'}$, $R^{1''}$ and/or $R^{1'''}$ = $-C(O)OCH_2OC(O)CH(CH_3)CH_3$. Only the trisubstituted derivative (Compound **9-1**) was tested. The trisubstituted compound demonstrated negative tail vein toxicity.

Example 10

Example 10 illustrates further modification of the above described prodrugs where the carboxylic acid group of the aspartic acid unit of the pseudomycin ring is modified to form a 3-monoamido derivative.

Synthesis of Compounds 10-1, 10-2, 10-3, 10-4 and 10-5:

$R^{1'}$, $R^{1''}$, $R^{1'''}$ = $-C(O)OCH_2OC(O)C(CH_3)_3$
R^2 = $-NHCH_2CH_2N(CH_3)_2$
R^3 = $-OH$

10-1

To a DMF solution (9 ml) of the prodrug 3-5 (864 mg, 0.52 mmol) was added 1-dimethylamino-2-aminoethane (57.9 µl, 0.52 mmol) and TBTU (168.6 mg, 0.52 mmol), followed by diisopropylethylamine (423 µl). After stirring at room temperature for 20 minutes, the reaction mixture was purified by reverse phase HPLC (ACN:0.1% TFA/Water). Lyophilization yielded 295 mg (34%) of Compound **10-1**.

$R^{1'}$, $R^{1''}$, $R^{1'''}$ = $-C(O)OCH_2OC(O)C(CH_3)_3$
R^2 = $-NH$(cyclopropyl)
R^3 = $-OH$

10-2

Compound **10-2** is synthesized using the same procedures as above except 0.052 mmol of cyclopropylamine is used in place of the 1-dimethylamino-2-aminoethane.

$R^{1'}$, $R^{1''}$, $R^{1'''}$ = $-C(O)OCH_2OC(O)C(CH_3)_3$
R^2 = $-NHCH_2(CO_2CH_3)$

R^3 = -OH

10-3

Compound **10-3** is synthesized using the same procedures as above except glycine methyl ester is used in place of the 1-dimethylamino-2-aminoethane.

$R^{1'}$, $R^{1''}$, $R^{1'''}$ = $-C(O)OCH_2OC(O)CH(CH_3)_2$
R^2 = $-NHCH_2CH_2N(CH_3)_2$
R^3 = -OH

10-4

Compound **10-4** is synthesized using the same procedures as above except 0.052 mmol of prodrug 7-1 is used in place of the prodrug 3-5.

$R^{1'}$, $R^{1''}$, $R^{1'''}$ = $-C(O)OCH_2OC(O)CH(CH_3)_2$
R^2 = -NH(cyclopropyl)
R^3 = -OH

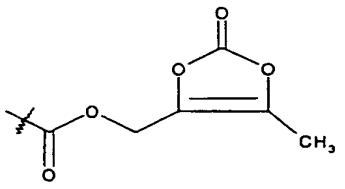
10-5

Compound **10-5** is synthesized using the same procedures as above except 0.052 mmol of prodrug 7-1 is used in place of the prodrug 3-5 and 0.052 mmol of cyclopropylamine is used in place of the 1-dimethylamino-2-aminoethane.

Example 11

Example 11 illustrates the formation of the prodrug of pseudomycin compounds where the carboxylic acid group of the aspartic acid unit of the pseudomycin ring has been modified to form a 3-amido derivative.

Synthesis of 3-monoamido derivative 11-1:



$R^{1'}$, $R^{1''}$ and $R^{1'''}$ =
R^2 = -NH(cyclopropyl)
R^3 = -OH

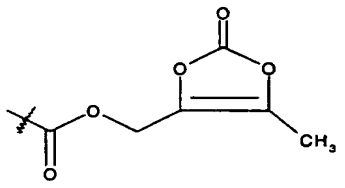
11-1

To a DMF solution (1 liter) containing Compound 2b-1 (1 eq.) was added 1.5 eq. of Compound 1a-4 and the mixture was stirred at room temperature for approximately 3 days. The solvent was partially removed and the residue purified by reverse-phase HPLC (Waters™, Delta Pak C18 column) to yield Compound 11-1 as well as the other mono- and di-substituted products.

Example 12

Example 12 illustrates the synthesis of a prodrug where the carboxylic acid group of both the aspartic acid and hydroxyaspartic acid units have been modified to form a bis-ester derivative.

Synthesis of Bis-ester 12-1:



$R^{1'}$, $R^{1''}$ and $R^{1'''}$ =
R^2 = $-OCH_3$
R^3 = $-OCH_3$

12-1

To a DMF solution (1 liter) containing Compound 3a-1 (1 eq.) was added 1.5 eq. of Compound 1a-4 and the mixture was stirred at room temperature for approximately 3 days. The solvent was partially removed and the residue purified by reverse-phase HPLC (Waters™, Delta Pak C18 column) to yield Compound 12-1 as well as the other mono- and di-substituted products.